# AN IMMUNOCHEMICAL DIFFERENCE BETWEEN MYOSINS FROM NORMAL AND HYPERTROPHIED RAT HEARTS

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## 1. Introduction

Myocardium hypertrophies in response to increase of cardiac work induced by mechanical overloading. In the hypertrophied heart, the velocity of shortening of the muscle [1] and the $Ca^{2+}$-dependent ATPase activity of myosin (reviewed in [2]) are depressed. The mechanism whereby mechanical overloading exerts its effects on muscle contractility is unknown. Several isozymes of myosin exist within the different muscles of the same animal species, and each of them exhibit a different $Ca^{2+}$-dependent activity [3]. In other respects, for a number of different muscles, $Ca^{2+}$-dependent activity of myosin is correlated with the velocity of shortening of the muscle [4,5]. Various authors hypothetized therefore that the alterations observed during mechanical overload could be due, at least in part, to the synthesis in the heart of an abnormal isozyme. Though extensive work has been devoted to this problem, evidence for a different molecule has not yet been clearly presented. Several observations, however, suggested subtle changes in the molecule [6–8].

We have investigated possible structural modifications of myosin during experimental hypertrophy of the rat heart using an immunochemical technique known for its ability to detect small structural changes [9], i.e., microcomplement fixation, MCF. Antibodies specific to myosin from normal rat heart were produced [10] by injected guinea pigs with heavy meromyosin, HMM, a fragment obtained by tryptic digestion of the molecule. Our results indicate that the antigenic structure of myosin from hypertrophied rat heart is modified, which strongly suggests that hypertrophy due to mechanical overloading induces an abnormal myosin species within the heart.

## 2. Materials and methods

Cardiac hypertrophy was induced in 20 male Wistar rats, 180–200 g, by constriction of the abdominal aorta followed, in 9 animals, by induction of aortic insufficiency 27–77 days later [11]. Abdominal aortic constriction was done with a 4/0 silk sutune over a needle 0.8 mm diam. (~50% stenosis). At various times after surgery (31–186 days), the surviving rats were killed by a blow on the head. The hearts were rapidly excised, the ventricles dissected free from the atria and bases of the great vessels, weighed ($V_w$) and frozen in liquid nitrogen within 2 min. For each animal, the theoretical ventricular weight ($Th_w$) was calculated according to a regression curve established in 80 controls: $Th_w$ = (202 × body wt) + 197. The % hypertrophy was $(V_w - Th_w) \times 100/Th_w$.

Rat cardiac proteins (crude extract, myosin and heavy-meromyosin) were prepared as in [12]. Depending on the experiment, either one heart or a pool of several hearts were used. Two series of 5 guinea-pigs were immunized with the same preparation of purified HMM [10] and for both series, the 5 individual sera from a particular bleeding were combined in equal proportions. Micro-complement fixation was done as in [9] in final vol. 0.7 ml. A fresh antiserum dilution was used for each experiment to avoid any possible effect of immunoglobulin denaturation on the MCF curves. $Ca^{2+}$- and $K^+$-dependent ATPase activities were determined as in [10].

## 3. Results

Immediate post-operative death was 10% after aortic stenosis alone, and 30% after the 2-step mechanical overloading. Twelve animals survived longer than 1 month. The % hypertrophy varied from one animal to another: 3 animals exhibited <30% hypertrophy; 5 from 30–80%; 4 from 80–112%. We found little if any macroscopic evidence of pulmonary edema or hepatic congestion. The data scattering we observed and the relatively limited extent of the hypertrophy point out one of the main difficulties of such studies, which is to obtain hypertrophies of a sufficient degree to be comparable to those commonly observed in humans (200–300%). Most data reported for animal models indicate hypertrophies ≤50% [2].

Figure 1 shows a typical experiment of complement fixation by crude tissular extracts and myosins

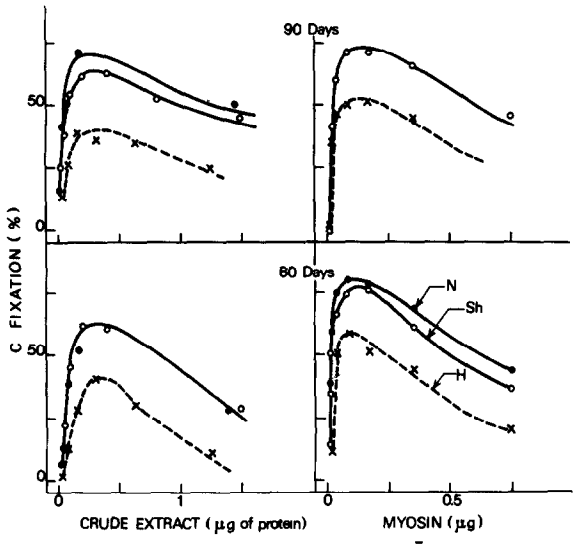


Fig.1. Microcomplement fixations with antisera to normal rat cardiac HMM and crude extracts (left) or myosins (right) obtained from the ventricles of normal (●——●, N), sham-operated (○——○, Sh) and hypertrophied (X – – – X, H) rat hearts. The antisera were pools of sera obtained from a series of 5 guinea pigs (series 27) and withdrawn 60 days (bottom) and 90 days (top) after initial immunization. Both controls were prepared out of 3 animals. As for the hypertrophic heart, only 1 animal was used, the degree of hypertrophy being 57%. The $Ca^{2+}$-ATPase activities were 1.0 μmol $P_i$ $min^{-1}$ $mg^{-1}$ for the normal, 1.1 for the sham and 0.7 for the hypertrophy. The $K^+$-dependent ATPase activities were, respectively, 0.41, 0.45 and 0.44 μmol $P_i$ $min^{-1}$ $mg^{-1}$.

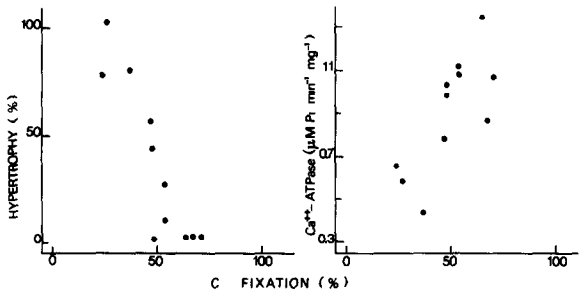


Fig.2. Relationship between % complement fixation of rat cardiac myosin and (i) the $Ca^{2+}$-dependent activity of the molecule (right); (ii) the degree of hypertrophy of the heart (left). The regression equations were: $y = 0.04\,X + 9.4, r = 0.76, p < 0.01$ (right); $y = -0.36\,X + 61.8, r = 0.86, p < 0.001$ (left).

from normal, sham-operated and hypertrophied rat hearts with antisera to normal rat cardiac HMM. The antisera were obtained from 2 different bleedings of the same series of guinea-pigs, at 2 months (fig.1, bottom) and (fig.1 top) 3 months after initial immunization. The patterns obtained with the 2 batches of antibodies were very similar. There was little if any difference between normal and sham-operated animals. In contrast, the amount of complement fixed by the hypertrophied heart was decreased by ~25%, which is far beyond experimental error [10]. The difference was apparent with the crude extracts (fig.1, left) and the myosins (fig.1, right), confirming that in this type of immune system, purified and unpurified myosins react equally [12]. The decrease of complement fixation was at the same antigen concentration for the 3 myosins, from 0.05–0.25 μg, thus evidencing a vertical shift without lateral displacement.

To test whether the antigenic difference could be evidenced with other antisera, a myosin prepared from an 80% hypertrophied heart was reacted with antisera prepared in 2 different series of guinea-pigs (not shown). Both antisera led to a significant decreased ability of myosin from hypertrophied as compared to controls to fix complement (from 70% and 62% to 40%). The difference between the two myosins appeared therefore sufficient enough to be detected by various antibodies producers.

Figure 2 summarizes the results for the 12 hypertrophied hearts and their controls. For each experiment complement fixation of myosin was plotted versus the $Ca^{2+}$-dependent ATPase activity of the

molecule (fig.2, right) or the degree of hypertrophy of the corresponding heart (fig.2, left). The enzymatic activity was significantly depressed, which is in full agreement with observations already reported in the rat [13] as well as in other species [6–8,14]. The decrease of the antigenic activity was significantly correlated with the decrease of the enzymatic activity, and with increase of heart weight, showing that the alterations of the structure and the function of the molecule occurred simultaneously during the development of myocardial hypertrophy.

## 4. Discussion

The vertical shift we observe (fig.1) reflects a decreased affinity for the antibodies, and therefore an altered recognition of some antigenic determinants on the molecule. According to [9], vertical shifts occur when only a minority of epitopes are modified. Numerous observations on globular and monomeric proteins (lysozymes and azurins) have shown that differences in the amount of complement fixed reflect differences in primary structure [15]. Such a relation is poorly documented for myosin, but the few available data suggest that, for this protein also, vertical shifts of the microcomplement fixation curves [10] indicate amino acid substitutions [16]. The most likely explanation for our results would be that the primary structures of normal and hypertrophied myosins diverge, and that a different population of myosin molecules was present within the hypertrophied heart. This is in agreement with the fact that marked differences in tertiary and quaternary structures were not found by others [6,8,13], and with the subtle changes postulated [8] after selective modifications of some thiol groups. Other observations suggest also slight alterations in the stoichiometry of the heavy and light chains [7] and in the thiol content of the molecule [17], but these findings remain controversial [8].

The presence of an abnormal myosin in hypertrophy due to mechanical overload is consistent with the changes in the myosin species already reported for the skeletal muscle in cross-innervation [18,19] or in chronic electric stimulation [20] experiments, and very recently for the heart in experimental hypertrophy induced by thyroxine intoxication [21]. Moreover the relation found between the increase of heart weight and the structural and enzymatic alterations (fig.2) indicates that the relative proportion of the abnormal myosin increases with the heart weight and suggests that normal and abnormal myosins coexist. This would be consistent with the simultaneous presence of two types of myosin in single muscle fibers during transformation induced by long-term stimulation [22]. The foregoing experimental evidence strongly suggests that we have found a second example of induction of a new myosin species within the heart.

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